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Grillotia similis (Linton, 1908) comb. n. (Cestoda: Trypanorhyncha) from Nurse Sharks in the Florida Keys

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ABSTRACT: Based on the examination of type material as well as light and scanning electron microscopy of voucher specimens collected from the nurse shark (*Ginglymostoma cirratum* (Bonnaterre, 1788)) in the Florida Keys, it was determined that *Grillotia simmonsi* Dollfus, 1969, is a junior synonym of *Rhynchobothrium simile* Linton, 1908. As the generic name *Rhynchobothrium* is no longer available, and the species is consistent with the diagnosis of *Grillotia* Guiart, 1927, the new combination *Grillotia similis* (Linton, 1908) is proposed and the description of this species is emended. Segment morphology is described and figured for the first time. The description of the vitellaria in the generic diagnosis of *Grillotia* is expanded to accommodate all of the species currently recognized in the genus. *Grillotia similis* was present in 81.8% of the 22 nurse sharks necropsied, with a mean intensity of 15.05 (±19.69) worms per host. The worms were found in the anteriormost 7 of the 16 spiral valve chambers and most abundantly in the anteriormost 3.

KEY WORDS: trypanorhynch, *Grillotia,* Lacistorhynchidae, nurse shark, Florida Keys.

Linton (1908) described *Rhynchobothrium simile* Linton, 1908, a species of relatively large trypanorhynch, from the nurse shark (*Ginglymostoma cirratum* (Bonnaterre, 1788)) off the Dry Tortuga Islands, west of the Florida Keys. Based on 3 immature specimens collected from the same host species off Sarasota, Florida by J. E. Simmons, Dollfus (1969) described *Grillotia simmonsi* Dollfus, 1969. Neither author was able to describe the segment morphology in any detail; however, Dollfus presented a very detailed account of the scolex morphology. Comparison of Linton's description and specimens with Dollfus' description and specimens leads us to believe that these species are synonymous. We recently collected numerous specimens of this species from nurse sharks in the Florida Keys. This additional material allows us to describe the segment morphology for the first time, to emend slightly the description of the tentacle armature, and to comment on the vitellaria in the genus *Grillotia* Guiart, 1927, in general. In addition, the exact location of this species within the spiral valve of the nurse shark was investigated.

Materials and Methods

During the summers of 1986 through 1988, 22 nurse sharks were examined for the presence and location of specimens of this large trypanorhynch species. Nurse sharks were caught on hook and line in the vicinity of Lower Matecumbe Key, in the Florida Keys, temporarily held in a floating wire pen, and pithed immediately prior to necropsy. The entire spiral valve was removed and cut along the dorsomedian line to expose the internal chambers. Each of the 16 chambers was examined separately for tapeworms. The tapeworms from each chamber were placed in a vial of AFA, such that there was 1 vial for each of the 16 chambers for each of the 22 nurse sharks.

Specimens for whole mounts were stained with Harris' hematoxylin or Semichon's acetocarmine, dehydrated in ethanol, cleared in xylene, and mounted in Canada balsam. The tentacles of 2 specimens were dissected away from the scolex musculature and stained and mounted as above. To circumvent the problem of viewing segment morphology through the dense circumcortical longitudinal musculature of this species, segments from 3 worms were cut longitudinally into dorsal and ventral halves using a razor blade. Each half was then stained and mounted as above. For cross sections, specimens were embedded in paraplast, sectioned at 10-μm intervals with an American Optics rotary microtome, stained in Gill's hematoxylin and eosin, dehydrated in ethanol, cleared in xylene, and mounted in Canada balsam. Specimens for scanning electron microscopy were hydrated, placed in 1% osmium tetroxide overnight, dehydrated in ethanol, critical point-dried in liquid CO_2, and mounted on stubs with double-sided adhesive tape and carbon paint, sputter-coated with gold, and examined with a Coates and Welter field emission scanning electron microscope.

The scolex terminology and hook numbering systems used follow those of Dollfus (1942, 1969, respectively). The genital terminology used follows that of Beveridge and Sakanari (1987). Measurements are in micrometers unless otherwise stated. The range is given for each numerical character, followed in parentheses by the mean, the standard deviation, the number of worms examined, and the total number of observations (when more than 1 structure per worm was examined). Illustrations were drawn with the aid of a drawing tube. Whole mounts of 10 voucher specimens were deposited in the U.S. National Museum Helminthological Collection (No. 80896) in Beltsville, Maryland. The sections from which Figures 1 and 2 were

drawn were deposited at the H. W. Manter Laboratory in Lincoln, Nebraska (No. 31166) as were the slides of tentacles isolated from their respective scolices (No. 31167), and whole mounts of 8 voucher specimens including the scolices from which the tentacle hooks in Figures 3 and 4 were drawn (No. 31165). Linton's specimens of *R. simile* (No. 8993) were borrowed from the U.S. National Museum Helminthological Collection. Two of Dollfus' syntype specimens of *G. simmonsi* were borrowed from the Laboratoire de Zoologie (Vers) at the Muséum National d'Histoire Naturelle in Paris.

Grillotia similis (Linton, 1908) comb. n. (Figs. 1–9)

The following information should emend Dollfus' (1969) description of the scolex of this species: Scolex unspined. Basal hooks on tentacles irregular in position; external surface of tentacle with group of 4 rose-thorn-shaped hooks of differing sizes arranged in a diamond pattern (Figs. 3, 4, 9).

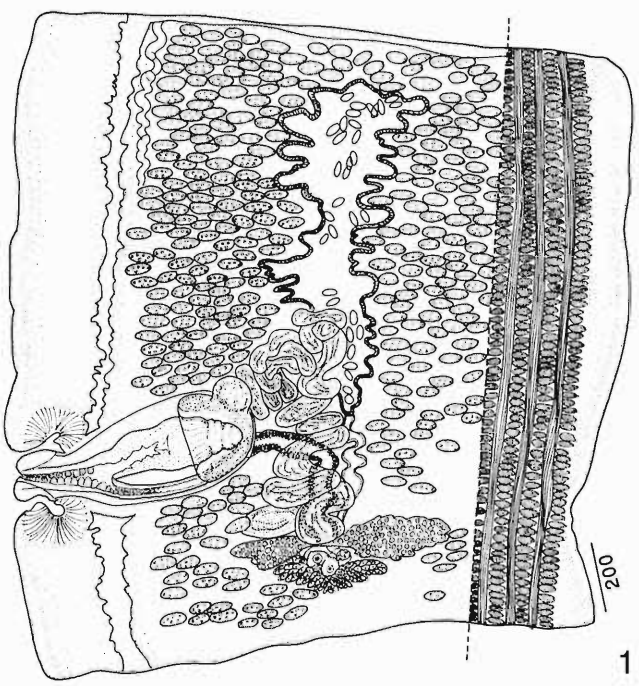
The following information should emend the descriptions of Linton (1908) and Dollfus (1969) for the strobila of this species: Worms 14.2–41.5 mm (28.1, 8.8, 22) long, maximum width 1,075–1,450 (1,240, 121, 22) occurring at bulbs. Strobila unspined. Neck lacking. Segments with numerous conspicuous bands of longitudinal muscle fibers. Immature segments wider than long; mature segments longer than wide, 550–1,725 (1,120.9, 297.4, 20) long by 375–1,500 (833.3, 285.7, 20) wide, ratio of length to width 0.52–2.95 (0.97, 0.44, 15, 31) acraspedote, apolytic. Genital atrium lateral, in posterior one-third of segment, 27.6–48.1% (34.8, 5.24, 16, 30) from posterior end, surrounded by sphincter-like muscle fibers. Hermaphroditic sac pyriform, thin-walled, 180–280 (221.5, 28.2, 5, 13) long by 490–670 (574, 47.7, 5, 13) wide; common genital duct of variable length, divides within hermaphroditic sac into vagina and cirrus. Cirrus unarmed, enters saccate internal seminal vesicle at proximal end. Small external seminal vesicle present; vas deferens greatly coiled, extending anteriorly then posteriorly along midline to ovarian isthmus. Testes numerous, 20–32.5 (24.8, 5.1, 20) long by 42.5–90 (62.1, 14.5, 20) wide, occupying medulla of entire segment median to excretory ducts, interrupted by hermaphroditic sac, 2–3 layers deep in cross section. Vagina piercing posterolateral wall of hermaphroditic sac, extending to midline of segment and then posterior to ovarian bridge. Ovary bilobed in dorsoventral view, 40–135 (101, 27.8, 8, 18) long by 320–650 (301.7, 216.3, 8, 18) wide, tetralobed in cross section. Mehlis' gland postovarian, between ovarian lobes, 55–130 (78.3, 21.7, 6, 10) long by 130–302.5 (220.2, 57.9, 6, 10) wide. Uterus medial, extending almost to anterior end of segment, with lateral branches; no uterine pore seen. Vitellaria follicular, circumcortical, arranged in longitudinal columns that alternate with bands of longitudinal muscle fibers. Eggs elipsoidal, 27.5–50 (40.9, 6.6, 20, 30) long by 17.5–23.7 (21.3, 1.9, 20, 30) wide, nonoperculated.

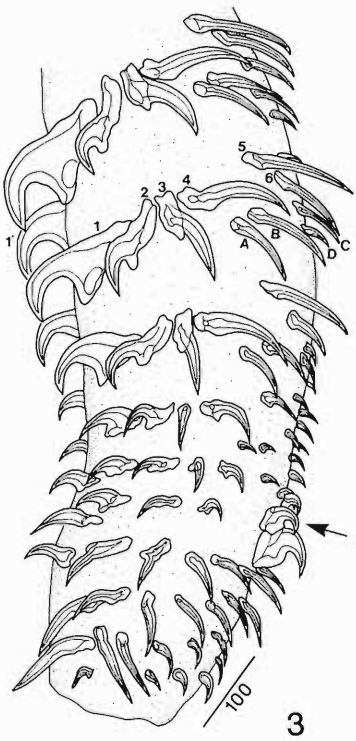
Remarks

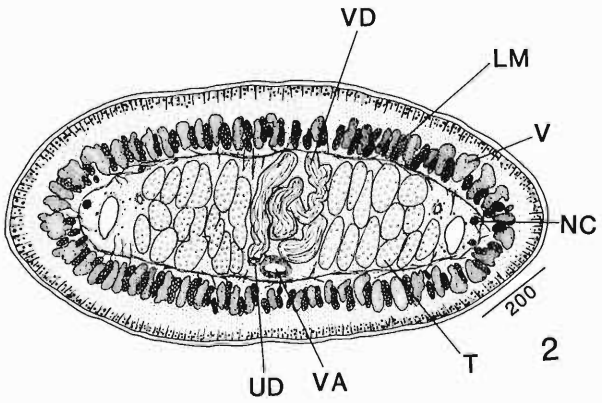
Comparison of Linton's specimens of *R. simile* (USNM No. 8993) and Dollfus' specimens of *G. simmonsi* confirms that they represent the same species. The distinctive basal group of 4 hooks (Figs. 3, 4, 9) was clearly visible on the tentacles of these specimens although they were not described by either Linton or Dollfus. As we first observed the presence of these hooks with scanning electron microscopy and then confirmed their presence with light microscopy, it is not surprising that these hooks were overlooked by these authors.

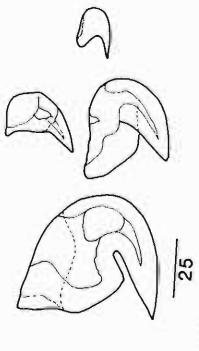
As the name *R. simile* predates the name *G. simmonsi,* and these 2 species appear to be synonymous, the correct specific epithet for this species should be "*simil.*" The name *Rhynchobothrium,* however, was rejected by Dollfus (1929) and therefore is no longer available. Based on characteristics of the tentacle armature, Dollfus (1969) placed his material into the genus *Grillotia,* in the monogeneric subfamily Grillotiinae, of the family Lacistorhynchidae. With the exception of the vitellaria, this species conforms to the diagnoses of *Grillotia* presented by Dollfus (1942), Yamaguti (1959), and more recently by Schmidt (1986), including the presence of a special basal group of small hooks on the external surface of the tentacles. With alteration of the gender of the specific epithet to match that of "*Grillotia,*" we propose the new combination *G. similis* (Linton, 1908) for this species.

At present there appears to be some confusion as to the configuration of the vitellaria in *Grillotia.* Dollfus (1942, p. 345) described the vitellaria of the genus as "á la fois en dedans et en dehors de la musculature longitudinal interne," and somewhat consistent with this characterization, Yamaguti (1959, p. 126) described the vitellaria as "medullary, intruding into cortex." Schmidt (1986, p. 64), however, described the vitellaria in this genus as "lateral, mostly med-



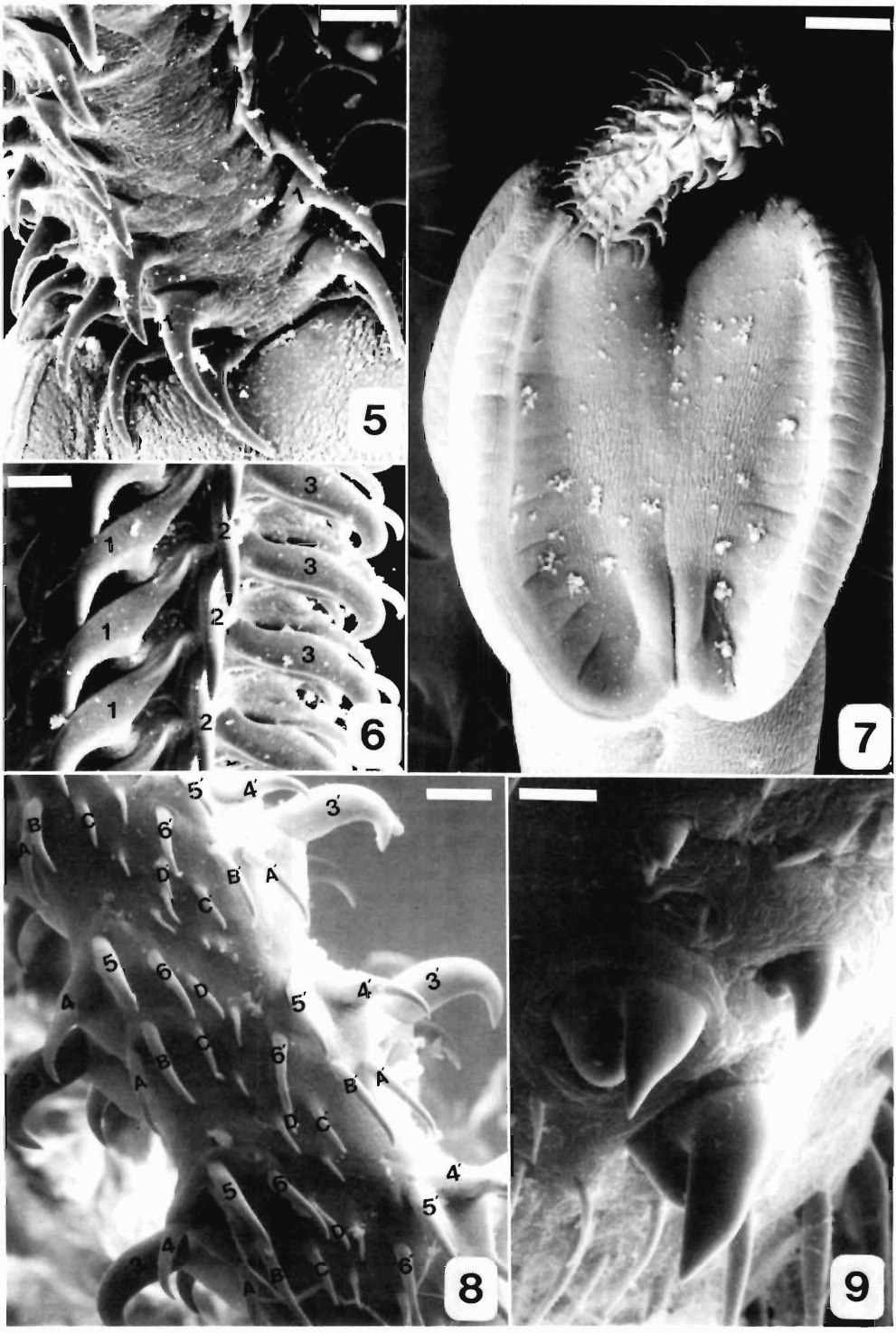






Figures 1, 2. ***Grillotia similis.*** **1. Mature proglottid from razor-blade section. Vitellaria and longitudinal muscle bundles are circumcortical but are drawn only to the reader's right of the dashed line. 2. Cross section through mature proglottid. Figure 3. Bothridial surface of tentacle showing location of distinctive diamond pattern of basal hooks on external surface (arrow). Figure 4. Enlarged view of distinctive basal hooks arranged as found on tentacle. VD—vas deferens, LM—longitudinal muscle bundle, V—vitellaria, NC—nerve cord, T—testis, VA—vagina, UD—uterine duct.**

ullary." Contrary to this latter characterization, cross sections of our specimens reveal vitellaria that encircle the segment, alternating with the longitudinal muscle bundles that divide the medullary region from the cortex (Fig. 2). As most of the 22 species of *Grillotia* (see Schmidt, 1986) are known only from larvae or immature adults, data on the vitellaria in other species of this genus are limited. Hart (1936) described vitellaria similar to that of *G. similis,* in *Grillotia musculara* (Hart, 1936) Dollfus, 1942, and Yamaguti (1959) published a cross section of *Grillotia erinaceus* (van Beneden, 1858) Guiart, 1927, illustrating this same configuration. But Hart (1936, p. 375)



Figures 5–9. Scanning electron micrographs of scolex of *Grillotia similis*. **5.** Internal surface of tentacle. Scale bar = 50 μm. Note hook-free region between hooks 1 and 1′. **6.** Metabasal hooks. Scale bar = 50 μm. Internal surface is to the left. **7.** Entire scolex. Scale bar = 200 μm. Note large posterior bothridial notch. **8.** Metabasal hooks on external surface of tentacle. Scale bar = 50 μm. Note the sinuous band of unlabeled small hooks

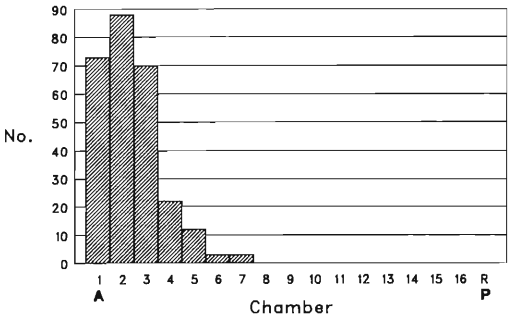


Figure 10. Distribution of 271 specimens of *G. similis* throughout the spiral valve chambers of 22 nurse sharks. Spiral valve chambers are numbered 1–16. R—rectum, A—anterior, P—posterior.

Table 1. Prevalence and intensity of *Grillotia similis* infections based on position in the gut in 22 nurse sharks.

Position (numbers indicate chambers of spiral valve)	Prevalence (%)	Intensity (mean no. worms/infected shark ± SD; range in parentheses)
1	59.1	5.6 ± 5.02 (1–17)
2	77.3	5.2 ± 5.21 (1–19)
3	54.5	5.8 ± 7.13 (1–24)
4	36.4	2.8 ± 2.9 (1–9)
5	27.3	2.0 ± 2.0 (1–6)
6	4.5	3.0 ± 0 (3)
7	13.4	1.0 ± 0 (1)
8–16	0.0	0.0
Rectum	0.0	0.0

also described vitellaria in *Grillotia megabothridia* (Hart, 1936) Dollfus, 1942, that "encircle the proglottids internal to the longitudinal muscle bundles" and are therefore medullary. In order to accommodate this variation we suggest that it would be best to describe the vitellaria of this genus as "encircling the segment, either internal to, or intermingled with the longitudinal muscle bundles dividing the cortex from the medulla."

The presence of a hermaphroditic sac in *G. similis* is worthy of some comment. A sac containing both the cirrus and the vagina, or a hermaphroditic sac, has been reported previously in *Lacistorhynchus* Pintner, 1913, *Mustelicolla* Dollfus, 1969, and *Callitetrarhynchus* Pintner, 1931 (see Beveridge and Sakanari, 1987; Campbell and Beveridge, 1988). Campbell and Beveridge consider this character to be of some taxonomic significance at the generic level. The segment morphologies of too few species of *Grillotia* have been examined in sufficient detail for the generality of this feature within the genus to be discussed; however, Dollfus (1942) described an organ that would appear to be a hermaphroditic sac (although he did not use that term) in *G. erinaceus* (van Beneden, 1858) Guiart, 1927, the type species of the genus.

Site specificity

The prevalence of *G. similis* in 22 nurse sharks was 81.8%. The overall mean intensity was 15.05 (±19.69) worms per infected shark with a range of 1–79. With respect to the 16 chambers of the nurse shark spiral valve, *G. similis* was restricted to the anterior 7, with a distinct preference for chambers 1–3. The spiral valve locations for the 271 specimens of *G. similis* recovered from 22 nurse sharks are given in Figure 10. Prevalence and mean intensity values, by spiral valve chamber, are given in Table 1. These results are consistent with the report of Linton (1908, p. 178) that, on 6 July 1906, 59 specimens of *R. simile* were found in "the upper part" of the spiral valve of a nurse shark.

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occupying the center of the external face of the tentacle. Compare to figure 19 in Dollfus (1969). 9. Distinctive diamond pattern of 4 hooks at base of external surface of tentacle. Scale bar = 20 μm. Hooks in Figures 5, 6, and 8 are numbered according to the system of Dollfus (1969).

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